

Antisense oligonucleotides against tenascin for the treatment of vitiligo

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The invention relates to specific, optionally modified oligonucleotides
5 having a length of up to 18 nucleotides, preferably a length of 7-15
nucleotides, which correspond to sections of tenascin-coding sequences
and can bind to these sequences, to their preparation and to the use
thereof, for example for the specific inhibition of the expression of tenascin
and for the production of medicaments which can be used for the treatment
10 of vitiligo.

Vitiligo is understood as meaning an acquired lack of melanocytes, by
means of which hypopigmented areas of skin result, which as a rule are
sharply demarcated and often symmetrically arranged, form one or two
15 spots or cover almost the entire skin. The hair in hypopigmented regions is
normally white and appears white even in the Wood light. The affected skin
sites are susceptible to sunburn. The cause of the disorder is unknown.
Although vitiligo is considered as a disease which is acquired in the course
of life, a familial cluster is occasionally found (autosomally dominant, with
20 incomplete penetrance and variable pronouncement). It can also follow an
unusual physical trauma, in particular a skull injury. The association of
vitiligo with Addison's disease, diabetes mellitus, pernicious anemia or
thyroid gland dysfunction and the increased occurrence of antibodies
against thyroglobulin, cells of the adrenal gland and border cells of the
25 stomach in the serum have led to an immunological or neurochemical
cause being suspected. Antibodies against melanin were found in some
patients.

All available therapeutic methods lead to satisfactory therapeutic results in
30 only some of the patients (F. Wach et al., H+G 71 (1996) 206). The present
therapies (S.P.W. Kumarasinghe, Ceylon Medical Journal 40 (1995) 94)
include photochemotherapies (PUVA) for example with methoxypsoralen,
phenylalanin or khellin, the transplantation of cultured melanocytes,
epidermal grafting, and treatment with steroids or placenta extracts.
35 Recently, treatment with pseudocatalase was reported (Schallreuter et al.,
Dermatology 190 (1995) 223). Small foci can also be covered with
cosmetic make-up or tannic acid solutions.

Poole et al. (British Journal of Dermatol. 137 (1997) 171) were able to show

that the vitiligo-affected skin has a high content of tenascin in comparison with normal skin. The high tenascin content can contribute to the loss of pigmentation and prevent repigmentation. Tenascin (Crossin, J. Cell. Biol. 61 (1996) 592) is an extracellular matrix glycoprotein, which consists of six identical subunits which are linked to the amino terminus via disulfide bridges. The tenascin subunits have a characteristic domain structure: a cysteine-rich sequence at the amino-terminal end is followed by three sequence sections, in each case constructed of repeating units, made of units homologous to EGF, of units homologous to fibronectin (type III) and of units homologous to fibrinogen.

A number of isoforms of the tenascin subunits exists (designated below as tenascin isoforms), which differ in the number of repeating units which are homologous to fibronectin type III. These isoforms are formed by alternative splicing of the tenascin pre-mRNA and subsequent translation of the various splice variants (A. Leprini et al., Perspectives in Developmental Neurobiology 2 (1994) 117-123). A cDNA from human tenascin was described (sequence in Table 1) by A. Siri et al. (Nucl. Acids Res. 19 (1991) 525-531). This cDNA is stored under the accession number X56160 in gene databases and can be obtained under this number, for example under EMBL/Genbank/DDBJ/NBRF-PIR. This cDNA contains a sequence section which codes for 12 repeating units which are homologous to fibrinogen type III. The cDNAs of the other isoforms of human tenascin are truncated in this sequence section and code for less than 12 of these repeating units.

The expression of tenascin is limited spatially and temporally and a significance is ascribed to it during the development of an organism and in pathological changes (Crossin, *vide supra*). Such pathological changes are, for example, vitiligo, tumors and inflammation.

Antisense oligonucleotides offer one possibility for the regulation of gene expression (E. Uhlmann and A. Peyman, Chemical Reviews 90, 543 (1990); S. Agrawal, TIBTECH 1996, 376). WO 94/21664 (L. Denner et al.) describes antisense oligonucleotides against tenascin, which are employed for the inhibition of the proliferation of the smooth cell musculature. The oligonucleotides described there have a length of at least 18 nucleotides.

It was an object of the present invention to make available novel oligonucleotides which have advantageous properties and which can be used for the complete and/or partial inhibition of the gene expression of tenascin.

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It has surprisingly been found that oligonucleotides which have a length of up to 18 nucleotides can effectively influence the expression of tenascin. The present invention relates to oligonucleotides having 7-17 nucleotide units which are optionally modified. In particular embodiments of the invention, the oligonucleotide has a length of 17, 16, 15, 14, 13, 12, 11, 10, 9, 8 or 7 nucleotides. The oligonucleotide corresponds to sections of tenascin-coding sequences (i.e. the oligonucleotide has a sequence which is complementary to the corresponding section of a tenascin-coding sequence) and the oligonucleotide binds specifically to this tenascin-coding sequence (nucleic acid), for example to the tenascin gene and/or tenascin mRNA and/or tenascin cDNA, the tenascin-coding sequence preferably being of human origin (e.g. human tenascin gene, human tenascin mRNA, human tenascin cDNA). The section of the tenascin-coding sequence which corresponds to the oligonucleotide or is complementary to the oligonucleotide preferably has a length of 17, 16, 15, 14, 13, 12, 11, 10, 9, 8 or 7 nucleotide units (this applies in particular to the determination of the length of a modified and/or chimeric oligonucleotide or of oligonucleotide analogs).

25 A particular embodiment of the invention relates to an oligonucleotide which binds to a nucleic acid which codes for one of the isoforms of human tenascin or parts thereof and inhibits its expression, where the oligonucleotide has a length of 7 to 15 nucleotides and can optionally be modified, and the physiologically tolerable salts of the oligonucleotide.

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A particular embodiment of the invention relates to an oligonucleotide which is directed against one or more specific regions of a tenascin-coding sequence, for example the translation start, the 5'-nontranslated region, the coding region and/or the 3'-noncoding region. In a particular embodiment of the invention, the oligonucleotide can also be directed against one or more regions of a tenascin-coding sequence which codes, for example, for certain domains of the tenascin, for example against the cysteine-rich domain, against a domain homologous to EGF, against a domain

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homologous to fibronectin type III and/or against a domain homologous to fibrinogen.

5 One embodiment of the invention relates to an oligonucleotide which binds to a nucleic acid which codes for one of the isoforms of human tenascin or parts thereof and inhibits its expression, where the oligonucleotide can bind to a region of the nucleic acid which comprises

- 10 a) a part of the 5'-noncoding region and/or the translation start or
- b) the translation start and/or a part of the coding region or
- c) a part of the coding region and/or a part of the 3'-noncoding region.

15 The invention relates in particular to an oligonucleotide which corresponds to a sequence section of the human cDNA according to SEQ ID NO. 1 (Table 1). The invention furthermore relates to an oligonucleotide which corresponds to a sequence section of the cDNA which is stored in gene databases under the accession number X56160.

20 In specific embodiments of the invention, an oligonucleotide can have, for example, one of the following sequences or parts thereof:

SEQ ID NO. 2: 3'-GGTTTGGGTGGAGGTGG -5'
 SEQ ID NO. 3: 3'-GGAGGTGGTACCCCCGG -5'
 SEQ ID NO. 4: 3'-GGTGGTACCCCCGG -5'
 SEQ ID NO. 5: 3'-GGAGGTGGTACCCC -5'
 SEQ ID NO. 6: 3'-AGAAAGAACGAAAGGAA -5'
 SEQ ID NO. 7: 3'-GGAGGTGGTACC -5'
 SEQ ID NO. 8: 3'-GGAGCGATGGCTTCCA -5'
 SEQ ID NO. 9: 3'-AAAGGAACGGGAGCG -5'
 SEQ ID NO. 10: 3'-GGTCGGTTTGGGTGG -5'
 SEQ ID NO. 11: 3'-CTTACAGGTCCGTTGA -5'
 SEQ ID NO. 12: 3'-GGCCGTGTTGCTGT -5'
 SEQ ID NO. 13: 3'-TCACCCCTCTTTCTGG -5'
 SEQ ID NO. 14: 3'-GGACACCGACACGG -5'
 SEQ ID NO. 15: 3'-AACGGGAGCGATGG -5'
 SEQ ID NO. 16: 3'-ATCTCGGGGTCGTC -5'
 SEQ ID NO. 17: 3'-AAAGAACGAAAGGAA -5'
 SEQ ID NO. 18: 3'-GGTGGTACCCC -5'
 SEQ ID NO. 19: 3'-CCCGGTACTGA -5' and
 SEQ ID NO. 20: 3'-CCACAGAAAGAAC -5'.

The sequences SEQ ID NO. 2 to SEQ ID NO. 20 correspond to sections of
 5 the tenascin-coding cDNA, as is shown in Table 1. An oligonucleotide
 which has one of the sequences SEQ ID NO. 2 to SEQ ID NO. 20 is
 complementary to a corresponding section of a tenascin-coding nucleic
 acid, e.g. a human tenascin cDNA, and can bind to this nucleic acid.
 Sequences SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 7
 10 and SEQ ID NO. 18 are examples of oligonucleotides which have a
 sequence which is directed against the translation start of the tenascin-
 coding sequences.

The invention also relates to derivatives of an oligonucleotide, for example
 15 its salts, in particular its physiologically tolerable salts. Physiologically
 tolerable salts are understood as meaning compounds which are readily
 soluble, soluble or poorly soluble in water, for example according to the
 definition in the "Deutsches Arzneibuch" [German Pharmacopeia] (9th

Edition 1986, official edition, Deutscher Apotheker Verlag Stuttgart), page 19. A specific embodiment of the invention relates to the sodium salt of the oligonucleotide according to the invention. Derivatives are also modified oligonucleotides.

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An oligonucleotide can be synthesized completely or partially from the natural nucleotides adenosine phosphate, guanosine phosphate, inosine phosphate, cytidine phosphate, uridine phosphate and thymidine phosphate. One embodiment of the invention relates to an oligonucleotide
10 which is synthesized from the natural nucleotides adenosine, guanosine, inosine, cytidine, uridine and thymidine and in which the nucleosides are linked to one another via phosphoric acid diester internucleoside bridges ("phosphoric acid diester bridges").

15 In other embodiments of the invention, an oligonucleotide can optionally contain one or more modifications, for example chemical modifications. An oligonucleotide can have a number of identical and/or different modifications. Modifications can be localized on certain nucleotide positions (nucleobase and/or β -D-2'-deoxyribose unit) and/or certain internucleoside
20 bridges.

Examples of chemical modifications are known to the person skilled in the art and are described, for example, in E. Uhlmann and A. Peyman, Chemical Reviews 90 (1990) 543 and "Protocols for Oligonucleotides and
25 Analogs" Synthesis and Properties & Synthesis and Analytical Techniques, S. Agrawal, Ed, Humana Press, Totowa, USA 1993, S.T. Crooke, F. Bennet, Ann. Rev. Pharmacol. Toxicol. 36 (1996) 107-129 and J. Hunziber and C. Leumann (1995) Mod. Synt. Methods, 7, 331-417.

30 The chemical modification of an oligonucleotide can mean, for example, a) the complete or partial replacement of the phosphoric acid diester bridges (internucleoside bridges) by modified phospho bridges, phosphorothioate, phosphorodithioate, $\text{NR}^1\text{R}^{1'}$ -phosphoramidate, boranophosphate, phosphate-($\text{C}_1\text{-C}_{21}$)-O-alkyl ester, phosphate-[($\text{C}_6\text{-C}_{12}$)-
35 aryl-($\text{C}_1\text{-C}_{21}$)-O-alkyl] ester, ($\text{C}_1\text{-C}_8$)alkylphosphonate and/or ($\text{C}_6\text{-C}_{12}$)-arylphosphonate bridges being examples of modified phospho bridges, where
 R^1 and $\text{R}^{1'}$ independently of one another are hydrogen, ($\text{C}_1\text{-C}_{18}$)-alkyl, ($\text{C}_6\text{-C}_{20}$)-aryl, ($\text{C}_6\text{-C}_{14}$)-aryl-($\text{C}_1\text{-C}_8$)-alkyl, preferably hydrogen, ($\text{C}_1\text{-C}_8$)-

alkyl and/or methoxyethyl, particularly preferably hydrogen, (C₁-C₄)-alkyl and/or methoxyethyl

or

R¹ and R^{1'}, together with the nitrogen atom carrying them, form a 5- to 6-

5 membered heterocyclic ring, which can additionally contain a further heteroatom from the group consisting of O, S and N;

and/or

b) the complete or partial replacement of the 3' and/or 5' phosphoric acid diester internucleoside bridges ("phosphoric acid diester bridges") by
10 "dephospho" bridges (described, for example, in Uhlmann, E. and Peyman, A. in "Methods in Molecular Biology", Vol. 20, "Protocols for Oligonukleotides and Analogs", S. Agrawal, Ed., Humana Press, Totowa 1993, Chapter 16, 355ff), formacetal, 3'-thioformacetal, methylhydroxylamine, oxime, methylenedimethylhydrazo, dimethylenesulfone and/or silyl
15 groups being examples of dephospho bridges;

and/or

c) the complete or partial replacement of the sugar phosphate backbone (replacement of sugar phosphate units) by other units, the other unit being
20 suitable, for example, to synthesize a "morpholine derivative" oligomer (described, for example, in E.P. Stirchak et al., Nucleic Acids Res. 17 (1989) 6129) (i.e. replacement by a morpholino derivative unit) and/or being suitable to synthesize a polyamide nucleic acid ("PNA") described, for example, in P.E. Nielsen et al., Bioconj. Chem. 5 (1994) 3 (EP 0 672 677) (i.e. replacement by a PNA unit, for example 2-aminoethylglycine) and/or being suitable to synthesize a phosphomonoacid ester
25 nucleic acid ("PHONA", "PMENA") (described, for example, in Peyman et al., Angew. Chem. Int. Ed. Engl. 35 (1996) 2632-2638, EP 0 739 898) (i.e. replacement by a PHONA unit);

and/or

30 d) the complete or partial replacement of the β -D-2'-deoxyribose (β -D-2'-deoxyribose unit) by modified sugar units, α -D-2'-deoxyribose, L-2'-deoxyribose, 2'-F-2'-deoxyribose, 2'-O-(C₁-C₆)alkylribose, preferably 2'-O-methylribose, 2'-O-(C₂-C₆alkenylribose, 2'-[O-(C₁-C₆) alkyl-O-(C₁-C₆alkyl)-ribose, 2'-NH₂-2'-deoxyribose, β -D-xylofuranose, α -arabinofuranose, 2,4-
35 dideoxy- β -D-erythrohexopyranose, carbocyclic sugar analogs (described, for example, in Froehler, J.Am.Chem.Soc. 114 (1992) 8320), open-chain sugar analogs (described, for example, in Vandendriessche et al., Tetrahedron 49 (1993) 7223) and bicyclo sugar analogs (described, for

analogues (described, for example, in M. Tarkov et al., *Helv. Chim. Acta* 76 (1993) 481) being examples of modified sugar units;

and/or

- e) the modification or the complete or partial replacement of the natural nucleoside bases by modified (nucleoside) bases ("nucleobases"), 5- (hydroxymethyl)uracil, 5-aminouracil, pseudouracil, dihydrouracil, 5-(C₁-C₆-alkyl)uracil, 5-(C₂-C₆)-alkenyluracil, 5-(C₂-C₆)alkynyluracil, 5-(C₁-C₆)alkylcytosine, 5-(C₂-C₆)alkenylcytosine, 5-(C₂-C₆)alkynylcytosine, 5-fluorouracil, 5-fluorocytosine, 5-chlorouracil, 5-chlorocytosine, 5-bromouracil, 5-bromocytosine, 7-deaza-7-substituted purines, 7-deaza-8-substituted purines, 8-azapurines, 2,4-diaminopurines, 5-bromocytosine, 5-bromouracil, 5-chlorocytosine, 5-chlorouracil, 5-fluorocytosine, 5-fluorouracil, hypoxanthine and uracil being examples of modified bases;
- and/or

- f) the conjugation to one or more molecules (oligonucleotide conjugates) which adapt the property(ies) of the oligonucleotide to specific requirements or favorably influence the properties (e.g. cell penetration, nuclease stability, affinity for the tenascin-coding target sequence, pharmacokinetics) of the oligonucleotide (e.g. antisense oligonucleotide, triple helix-forming oligonucleotide) and/or in the hybridization of the oligonucleotide on the target sequence can attack this with binding and/or crosslinking, polylysine, intercalators such as pyrene, acridine, phenazine, phenanthridine, fluorescent compounds such as fluorescein, crosslinkers such as psoralen, azidoproflavine, lipophilic molecules such as (C₁₂-C₂₀)alkyl, lipids such as 1,2-dihexadecyl-rac-glycerol, steroids such as cholesterol, testosterone, vitamins such as vitamin E, poly- or oligoethylene glycol, (C₁₂-C₁₈)alkyl phosphate diesters and -O-CH₂-CH(OH)-O-(C₁₂-C₁₈)-alkyl being examples of molecules which can be conjugated to an oligonucleotide, where such molecules can be conjugated to the oligonucleotide at the 5' and/or at the 3' end and/or within the sequence, e.g. via a nucleobase;

and/or

- g) the conjugation to a 2'5'-linked oligoadenylate or a derivative thereof, a 2'5'-linked triadenylate, a 2'5'-linked tetraadenylate, a 2'5'-linked pentaadenylate etc. being examples of 2'5'-linked oligoadenylates and cordycepin (2'5'-linked 3'-deoxyadenylate) being an example of a derivative of a 2'5'-linked oligoadenylate, the conjugation preferably taking place via a linker, where the 5'-end of the 2'5'-linked oligoadenylate can preferably be a phosphate, diphosphate or triphosphate group, where the linker, for

example, can be an oligoethylene glycol, triethylene glycol, tetraethylene glycol and hexaethylene glycol being examples of oligoethylene glycol linkers;
and/or

- 5 h) the introduction of a 3'-3' and/or 5'-5' inversion at the 3' and/or at the 5' end of the oligonucleotide, this type of chemical modification being known to the person skilled in the art and being described, for example, in M. Koga et al., J. Org. Chem. 56 (1991) 3757.

- 10 In preferred embodiments of the invention, the oligonucleotide has one or more chemical modifications which independently of one another are selected from

- a) the complete or partial replacement of the phosphoric acid diester bridges by phosphorothioate and/or (C₁-C₈)alkylphosphonate bridges,
- 15 b) the complete or partial replacement of the sugar phosphate backbone by PNA units and/or PHONA units,
- c) the complete or partial replacement of the β -D-2'-deoxyribose units by 2'-F-2'-deoxyribose, 2'-O-(C₁-C₆)alkylribose and/or 2'-[O-(C₁-C₆)alkyl-O-(C₁-C₆)alkyl]ribose,
- 20 d) the complete or partial replacement of the natural nucleoside bases by 5-(C₂-C₆)-alkynyluracil and/or 5-(C₂-C₆)alkynylcytosine,
- e) the conjugation of the oligonucleotide to one or more molecules which independently of one another can be selected from the group comprising lipophilic molecules, e.g. (C₁₂-C₂₀)alkyl, lipids, e.g. 1,2-
- 25 dihexadecyl-rac-glycerol, steroids, e.g. cholesterol and/or testosterone, vitamins, e.g. vitamin E, poly- or oligoethylene glycol, (C₁₂-C₁₈)-alkyl phosphate diesters and-O-CH₂-CH(OH)-O-(C₁₂-C₁₈)-alkyl and
- f) one or more 3'-3' inversions at the 3' end of the oligonucleotide,

- In another preferred embodiment of the invention, the oligonucleotide has
30 one or more chemical modifications which independently of one another can be selected from the group comprising

- a) the complete or partial replacement of the phosphoric acid diester bridges (phosphodiester bridges) by phosphorothioate bridges,
- b) the complete or partial replacement of the β -D-2'-deoxyribose units
- 35 by 2'-F-2'-deoxyribose, 2'-O-(C₁-C₆)alkylribose and/or 2'-[O-(C₁-C₆)alkyl-O-(C₁-C₆)alkyl]ribose,
- c) conjugation to lipophilic molecules, e.g. (C₁₂-C₂₀)-alkyl, to lipids, e.g. 1,2-dihexadecyl-rac-glycerol, to (C₁₂-C₁₈)alkyl phosphate diesters and/ or to -O-CH₂-CH(OH)-O-(C₁₂-C₁₈)-alkyl.

Processes for the preparation of an oligonucleotide conjugate are known to the person skilled in the art and are described, for example, in Uhlmann, E. & Peyman, A., Chem. Rev. 90 (1990) 543 and/or M. Manoharan in
5 "Antisense Research and Applications", Crooke and Lebleu, Eds., CRC Press, Boca Raton, 1993, Chapter 17, p.303ff. and/or EP-A 0 552 766.

In a particular embodiment of the invention, an oligonucleotide is made available which can have one or more modifications and which has one of
10 the sequences SEQ ID NO. 2 - SEQ ID NO. 20 or which corresponds to one of the sequences SEQ ID NO. 2 to SEQ ID NO. 20 or which corresponds to the appropriate sequence sections of a tenascin-coding sequence and can bind to this section of the tenascin-coding sequence.

15 In a particular embodiment of the invention, oligonucleotide is made available in whose sequence each nucleotide (base and/or sugar and/or internucleoside bridge) is modified. In a particular embodiment of the invention, for example, the oligonucleotide is completely synthesized from phosphorothioates (universally modified phosphorothioate, all
20 internucleoside bridges modified). In a further specific embodiment of the invention, an oligonucleotide is made available which corresponds to one of the sequences SEQ ID NO. 2 - SEQ ID NO. 20, but where the phosphodiester bridges between the individual nucleosides (i.e. the internucleoside bridges between the individual nucleosides) are completely
25 replaced by phosphorothioate bridges (i.e. phosphorothioate groups between the nucleosides).

In a further particular embodiment of the invention, an oligonucleotide is made available by only replacing some of the phosphodiester bridges by
30 phosphorothioate bridges. In particular, the invention comprises oligonucleotides which are only minimally (or partially) modified. The principle of the minimally modified oligonucleotides is described in A. Peyman, E. Uhlmann, Biol. Chem. Hoppe-Seyler, 377 (1996) 67-70. In this case, 1-5, preferably 1-3 terminal nucleotide units (preferably the
35 corresponding internucleoside bridges) at the 5' and/or at the 3' end and, if appropriate, additionally selected internal pyrimidine positions or preferably the corresponding internucleoside bridges which are located at the 3' and/or 5' end of the corresponding pyrimidine nucleoside, are modified or replaced, internucleoside bridges preferably being replaced by

phosphorothioate bridges. Oligonucleotides minimally modified in this way have particularly advantageous properties, for example they exhibit particular nuclease stability on minimal modification.

- 5 A particular embodiment of the invention relates to an oligonucleotide in which selected internucleoside bridges are replaced by modified internucleoside bridges, preferably by phosphorothioate bridges.

The invention relates to an oligonucleotide in which either

- 10 a) only certain phosphodiester internucleoside bridges or
b) all phosphodiester internucleoside bridges
are modified.

- The invention furthermore relates to an oligonucleotide in which 1 – 5 terminal internucleoside bridges are modified at the 5' and/or at the 3' end of the oligonucleotide. The invention also relates to an oligonucleotide in
15 which the internucleoside bridges located at the 3' and/or 5' end of nonterminal nucleosides which contain a pyrimidine base (internal pyrimidine nucleosides) are modified.

- 20 Specific embodiments of the invention comprise a minimally modified oligonucleotide which has one of the sequences selected from the group consisting of the sequences SEQ ID NO. 21 to SEQ ID NO. 39,
where

SEQ ID NO. 21: is 3'- GsGsTsTsTGGGTsGGAGGsTsGsG -5',
 SEQ ID NO. 22: is 3'- GsGsAsGGTsGGTsACsCCsCCsGsG -5',
 SEQ ID NO. 23: is 3'- GsGsTGGTsACsCsCCsCsGsG -5',
 SEQ ID NO. 24: is 3'- GsGsAGGTsGGTsACsCsCsC -5',
 SEQ ID NO. 25: is 3'- AsGsAAAGAAcCsGAAAGGsAsA -5',
 SEQ ID NO. 26: is 3'- GsGsAGGTsGGTsAsCsC -5',
 SEQ ID NO. 27: is 3'- GsGsAGCsGATsGGCsTsTsCsCsA -5',
 SEQ ID NO. 28: is 3'- AsAsAGGAACsGGGAGsCsG -5',
 SEQ ID NO. 29: is 3'- GsGsTCGGTsTsTGGGTsGsG -5',
 SEQ ID NO. 30: is 3'- CsTsTACAGGTsCsCGTsTsGsA -5',
 SEQ ID NO. 31: is 3'- GsGsCsCGsTGTsTCGCsTsGsT -5',
 SEQ ID NO. 32: is 3'- TsCsACsCCsCTsCsTTsTsCsTsGsG -5',
 SEQ ID NO. 33: is 3'- GsGsAsCACsCGACsACsGsG -5',
 SEQ ID NO. 34: is 3'- AsAsCsGGGAGCGATsGsG -5',
 SEQ ID NO. 35: is 3'- AsTsCsTCGGGGTsCsGsTsC -5',
 SEQ ID NO. 36: is 3'- AsAsAGAACsGAAAGGsAsA -5',
 SEQ ID NO. 37: is 3'- GsGsTGGTsACsCsCsC -5',
 SEQ ID NO. 38: is 3'- CsCsCsGGTsACsTsGsA -5',
 SEQ ID NO. 39: is 3'- CsCsAsCAGAAAGsAsAsC-5' and

5 "s" indicating the position of a modified internucleoside bridge or dephospho bridge, "s" preferably indicating the position of a phosphorothioate bridge.

10 The sequences SEQ ID NO. 21 to SEQ ID NO. 39 correspond to the sequences SEQ ID NO. 2 - SEQ ID NO. 20, i.e. they can bind to the same regions of a tenascin-coding sequence, where, however, in contrast to the SEQ ID NO. 2-20, some of the phosphodiester bridges are replaced by modified phosphodiester bridges or dephospho bridges, preferably by phosphorothioate bridges (in the sequence marked by an "s").

15 A further embodiment of the invention relates to chimeric oligonucleotides. A chimeric oligonucleotide is synthesized from at least two different sequence sections, for example from a DNA section and a modified section, e.g. a PNA section and/or a PHONA section. These different sections impart particular properties to the entire oligonucleotide.

A particular form of chimeric oligonucleotides is described, for example, in Matteucci and Wagner, Nature 384 SUPP (1996) 20-22. A chimeric oligonucleotide can contain, for example,

1. a so-called core sequence, which consists of approximately seven
5 nucleotides and which can activate the RNase H, and
2. one or more flanking sequences which increase the affinity, specificity
and/or nuclease stability of the oligonucleotide.

For example, the core sequence can have internucleoside bridges modified
in certain positions, for example the core sequence can contain
10 phosphorothioate and/or phosphodiester bridges. Suitable flanking
sequences are, for example, sequences in which the sugar phosphate
backbone (replacement of one or more sugar phosphate units) and/or β -D-
2'-deoxyribose units are replaced. Suitable flanking sequences are, for
example, PNAs and/or 2'-O-alkyl derivatives such as, for example, 2'-O-
15 methyl and/or 2'-O-propyl and/or 2'-methoxyethoxy derivatives.

A particular embodiment of the invention relates to a chimeric
oligonucleotide which has one of the sequences SEQ ID NO. 40 - SEQ ID
NO. 58, where

- 20 x independently of one another represents an unmodified or a
modified phosphodiester internucleoside bridge or a dephospho
bridge, preferably phosphorothioate and/or phosphorus diester
and
- y independently of one another represents the replacement of a sugar
25 phosphate unit or a β -D-2'-deoxyribose unit, preferably 2'-O-methyl-,
2'-O-propyl- and/or 2'-methoxyethoxyribose or a PNA unit,
where

SEQ ID NO. 40: is 3'- GyGyTyTyTyGxGxGxTxGxGxGxGyGyTyGyG -5',
 SEQ ID NO. 41: is 3'- GyGyAyGyGyTxGxGxTxAxGxGxGxCyCyGyG -5',
 SEQ ID NO. 42: is 3'- GyGyTxGxGxTxAxGxGxGxCyCyGyG -5',
 SEQ ID NO. 43: is 3'- GyGyAyGyGxTxGxGxTxAxGyCyCyC -5',
 SEQ ID NO. 44: is 3'- AyGyAyAxGxGxGxGxGxGxGyGyAyA -5',
 SEQ ID NO. 45: is 3'- GyGyAxGxGxTxGxGxTxGyCyC -5',
 SEQ ID NO. 46: is 3'- GyGyAxGxCxGxGxTxGyGyCyTyTyCyCyA -5',
 SEQ ID NO. 47: is 3'- AyAyAyGxGxGxGxGxGyGyAyGyCyG -5',
 SEQ ID NO. 48: is 3'- GyGyTyCxGxGxTxTxGxGyGyTyGyG -5',
 SEQ ID NO. 49: is 3'- CyTyTyAxGxGxGxTxGxGyTyTyGyA -5',
 SEQ ID NO. 50: is 3'- GyGyCyCxGxGxTxTxGxGyCyTyGyT -5',
 SEQ ID NO. 51: is 3'- TyCyAyCxGxGxTxGxTxTyTyCyTyGyG -5',
 SEQ ID NO. 52: is 3'- GyGyAyCxGxGxGxGxGxGxGyCyGyG -5',
 SEQ ID NO. 53: is 3'- AyAyCyGxGxGxGxGxGxGxGyTyGyG -5',
 SEQ ID NO. 54: is 3'- AyTyCyTxGxGxGxGxGxTxGyTyC -5',
 SEQ ID NO. 55: is 3'- AyAyAyGxGxGxGxGxGxGyGyAyA -5',
 SEQ ID NO. 56: is 3'- GyGyTxGxGxTxAxGxCyCyC -5',
 SEQ ID NO. 57: is 3'- CyCxGxGxGxTxAxGyTyGyA -5',
 SEQ ID NO. 58: is 3'- CyCyAxGxGxGxGxGxGyAyAyC -5'

The sequences SEQ ID NO. 40 - SEQ ID NO. 58 correspond to the
 abovementioned sequences SEQ ID NO. 2 to SEQ ID NO. 20, i.e. they
 5 bind to the corresponding sequence sections of a tenascin-coding
 sequence, where, however, the modifications mentioned are present.

The invention relates to processes for the preparation of the
 oligonucleotides. The oligonucleotides described can be prepared with the
 10 aid of various known, chemical processes, e.g. applying the standard
 phosphoramidite chemistry using iodine or TED (tetraethylthiuram disulfide)
 as oxidant. This process is described, for example, in Eckstein, F. (1991)
 "Oligonucleotides and Analogues, A Practical Approach", IRL Press,
 Oxford. The oligonucleotides can also be prepared by processes which
 15 optionally contain one or more enzymatic steps.

The invention relates to the use of the oligonucleotides. The
 oligonucleotides can be used for hybridization or binding to tenascin-coding
 (single-stranded and/or double-stranded) nucleic acids, for example DNA

relates to the use of the oligonucleotides for hybridization with or binding to nucleic acids which have the sequence SEQ ID NO. 1 according to Table 1 or with nucleic acids which have parts of this sequence (for example sequences which code for tenascin isoforms) or with nucleic acids whose
5 sequence differs slightly from these sequences (which have, for example, one or more point mutations).

The invention furthermore relates to the use of the oligonucleotides for the modulation and for the complete or partial inhibition of the expression of
10 tenascin or various tenascin isoforms or of mutants thereof, for example for the complete or partial inhibition of transcription and/or of translation.

The invention relates, for example, to the use of the oligonucleotides as antisense oligonucleotides. Moreover, the oligonucleotides can be used as
15 aids in molecular biology.

The invention furthermore relates to the use of the oligonucleotides as pharmaceutical and/or diagnostic or the use of the oligonucleotides for the production of pharmaceuticals and/or diagnostics. In particular, the
20 oligonucleotides can be employed in pharmaceuticals which are suitable for the prevention and/or treatment of diseases which accompany the expression of an overexpression of tenascin. Since the expression of tenascin is normally, i.e., for example, in the healthy person, limited spatially and temporally, a deviation from this normal spatial and temporal
25 expression can be regarded as overexpression. Furthermore, the oligonucleotides can be employed in diagnostic processes. Such diagnostic processes can be employed, for example, for the diagnosis or early recognition of diseases which accompany abnormal expression (e.g. overexpression) of tenascin.

30 The invention also relates to a test kit which contains one or more oligonucleotides according to the invention and, if appropriate, further components. Such a test kit can be employed, for example, in diagnosis and as a precaution, for example against skin cancer disorders..

35 The invention further relates to the use of the oligonucleotides or of pharmaceuticals which contain these oligonucleotides for the treatment of diseases in which tenascin or an overexpression of tenascin is the cause or is involved.

The invention relates in particular to the use of the oligonucleotides or of pharmaceuticals which contain these oligonucleotides for the treatment and/or prevention of diseases in which a dysregulation or disorder of the immigration or of the presence or of the inclusion of melanocytes in epithelial cell layers, for example in the epithelial cell layer of the epidermis, of the choroid membrane of the eye or of the substantia nigra as the basis serves or is involved and of Addison's disease, diabetes mellitus, pernicious anemia and/or thyroid gland dysfunctions.

10

The invention relates in particular to the use of the oligonucleotides or of pharmaceuticals which contain these oligonucleotides for the treatment and/or prevention of vitiligo and other depigmentation diseases or depigmentation disorders (e.g. of the skin, hair, eyes) for example albinism and/or for the treatment of psoriasis and/or for the treatment of cancer, e.g. for the inhibition of tumor growth and tumor metastasis, for example in melanomas and/or for the treatment of inflammations, in particular as antiinflammatories and/or for the treatment and/or prophylaxis of cardiovascular disorders, for example of restenosis.

20

In particular, the invention relates to the use of the oligonucleotides for the treatment of vitiligo or for the production of pharmaceuticals which can be used for the treatment of vitiligo. The invention moreover relates quite generally (i.e. also oligonucleotides having a length of greater than or equal to 18 nucleotides) to the use of oligonucleotides for the treatment of vitiligo or the production of pharmaceuticals which can be used for the treatment of vitiligo.

25

The invention furthermore relates to the use for the treatment of vitiligo in combination with known therapeutic processes, for example in combination a) with photochemotherapy (PUVA), e.g. using methoxypsoralen, phenylalanine and/or khellin and/or b) with the transplantation of cultured melanocytes (epidermal grafting) and/or c) with a steroid treatment and/or d) with a treatment with placenta extracts and/or e) with a treatment with pseudocatalase.

30

35

The invention furthermore relates to processes for the production of pharmaceuticals (pharmaceutical preparations). For the production of pharmaceuticals, one or more different oligonucleotides or their

physiologically tolerable salts are mixed, it optionally being possible to add further pharmaceutical vehicles and/or additives.

5 The invention furthermore relates to pharmaceutical preparations (pharmaceuticals), which contain one or more different oligonucleotides and/or their physiologically tolerable salts, and, if appropriate, pharmaceutical vehicles and/or additives.

10 The oligonucleotide(s) and/or its/their physiologically tolerable salts can be administered to animals, preferably to mammals, in particular to humans as pharmaceuticals on its/their own, in mixtures with one another or in the form of pharmaceutical preparations. The pharmaceuticals can make possible topical, percutaneous, parenteral and/or enteral administration. The administration form preferred in each case depends on the specific
15 conditions in each case. For the treatment of vitiligo, for example, a topical application, e.g. in the form of ointments, lotions or tinctures, emulsions or suspensions, is preferred. Likewise, the frequency of the administration depends on the individual conditions. For the treatment of vitiligo, for example, a topical composition can be applied to the depigmented skin site
20 one to two times during the day.

As active constituent, pharmaceuticals or pharmaceutical preparations can contain an efficacious dose of at least one oligonucleotide and/or a mixture of a number of oligonucleotides and, if appropriate, additional,
25 pharmaceutically innocuous vehicles and/or additives. Pharmaceutical preparations can contain approximately 0.1% (percent by weight) or less up to approximately 90% (percent by weight) or more of the therapeutically active oligonucleotide or the pharmaceutically active oligonucleotide.

30 The pharmaceutically efficacious dose of the respective oligonucleotide or of an oligonucleotide which is a constituent of a mixture of various oligonucleotides can vary within wide limits and is to be adapted to the individual conditions in each individual case.

35 The production of the pharmaceutical preparations can be carried out in a manner known per se, e.g. described in Remingtons Pharmaceutical Sciences (1985), Mack Publ. Co., Easton, PA., it optionally being possible to use pharmaceutically inert inorganic and/or organic vehicles. For the production of pills, tablets, coated tablets and/or hard gelatin capsules, it is

possible to use, for example, lactose, cornstarch and/or derivatives thereof, talc, stearic acid and/or its salts. Vehicles which can be used for soft gelatin capsules and/or suppositories are, for example, fats, waxes, semisolid and/or liquid polyols, natural and/or hardened oils. Vehicles which can be used for the production of solutions and/or syrups are, for example, water, sucrose, invert sugar, glucose and/or polyols. Vehicles which can be used for the production of injection solutions are, for example, water, alcohols, glycerol, polyols and/or vegetable oils. Vehicles which can be used for microcapsules, implants and/or rods are, for example, copolymers, e.g. of glycolic acid and lactic acid. Moreover, liposome formulations which are known to the person skilled in the art (N. Weiner, Drug Develop Ind Pharm 15 (1989) 1523; "Liposome Dermatics, Springer Verlag 1992), for example HVJ liposomes (Hayashi, Gene Therapy 3 (1996) 878) are suitable. Dermal administration can be carried out, for example, also with the aid of ionophoretic methods and/or with the aid of electroporation. Moreover, lipofectins and/or other (nucleic acid or DNA) carrier systems, for example those which are used in gene therapy, can be used. In particular, suitable systems are those with whose aid oligonucleotides can be introduced into eukaryotic cells or the nuclei of eukaryotic cells with great efficiency.

In addition to the active compounds and vehicles, a pharmaceutical preparation can additionally contain additives, such as, for example, fillers, extenders, disintegrants, binding agents, lubricants, wetting agents, stabilizers, emulsifiers, preservatives, sweeteners, colorants, flavorings or aromatizers, thickening agents, diluents, buffer substances, furthermore solvents and/or solubilizers and/or agents for achieving a depot effect, and salts for changing the osmotic pressure, coating agents and/or antioxidants. They can also contain two or more different oligonucleotides and/or their physiologically tolerable salts and furthermore, in addition to at least one oligonucleotide, one or more other therapeutically active substances.

Examples

Example 1: Oligonucleotide synthesis

The oligonucleotide was synthesized on an automatic DNA synthesizer (Applied Biosystems Model 380B or 394) using the standard phosphoramidite chemistry and oxidation with iodine (F. Eckstein, Ed

"Oligonucleotides and Analogs, A Practical Approach", IRL Press, Oxford, 1991). For the introduction of phosphorothioate bridges in mixed phosphorothioates and phosphodiester oligonucleotide, oxidation was carried out with TETD (tetraethylthiuram disulfide) instead of iodine (Applied Biosystems User Bulletin 65). After removal of solid carrier (CPG or Tentagel) and removal of the protective groups with conc. NH_3 at 55°C (18 h), the oligonucleotide was first purified by butanol precipitation (Sawadogo, Van Dyke, Nucl. Acids Res. 19 (1991) 674). The sodium salt was then obtained by precipitation from a 0.5 M NaCl solution using 2.5 parts by volume of ethanol.

The oligonucleotide was analyzed with the aid of

a) analytical gel electrophoresis (gel: 20% acrylamide, 8M urea; running buffer: 454M tris borate buffer, pH 7.0) and/or

b) HPLC analysis (column material: Waters GenPak FAX; gradient: CH_3CN (400 ml), H_2O (1.6 l), NaH_2PO_4 (3.1 g), NaCl (11.7 g) pH 6.8 (0.1 M in NaCl) after CH_3CN (400 ml), H_2O (1.6 l), NaH_2PO_4 (3.1 g), NaCl (175.3 g), pH 6.8 (1.5 M in NaCl)) and/or

c) capillary gel electrophoresis (Beckmann capillary eCAPTM, U100P gel column, 65 cm length, 100 mm I.D., window 15 cm from one end; buffer: 140 μM tris, 360 mM boric acid, 7M urea) and/or

d) electrospray mass spectroscopy.

The analysis of the oligonucleotide showed that this was in each case present in a purity of greater than 90%. The methods for the analysis of oligonucleotides are described, for example, in Schweiber and Engler "Analysis of oligonucleotides" (in "Antisense – from technology to therapy", a laboratory manual and textbook, Schlingensiepen et al. eds., Biol. Science, Vol. 6 (1997) p. 78-103).

Synthesized oligonucleotide:

ODN1 (sequence SEQ ID NO. 24): 3'-GsGsAGGTsGGTACsCsCsC-5'

Example 2: Production of a pharmaceutical preparation

50 mg of ODN 1 from Example 1 can be closely mixed with 1 g of Dermatop® (Hoechst Aktiengesellschaft, Frankfurt am Main, Germany) base cream and the mixture stored at temperatures of $<10^{\circ}\text{C}$.

5

Example 3:

The cream from Example 2 can then be applied twice daily (in the morning and afternoon or evening) to a depigmented skin site of a vitiligo patient.

Table 1: Sequence SEQ ID NO. 1:

Sequence of the human tenascin cDNA according to A. Siri et al. Nucl. Acids Res. 19 (1991) 525-531.

GAATTCGCTA GAGCCCTAGA GCGCCAGCAG CAGCCAGCCA AACCCACCTC CACCATGGGG	60
GCCATGACTC AGCTGTTGGC AGGTGTCTTT CTTCCTTTTC TTGCGCTGGC TACCGAAGGT	120
GGGGTCCTCA AGAAAGTCAT CCGGCACAAG CGACAGAGTG GGGTGAACGC CACCCTGCCA	180
GAAGAGAACC AGCCAGTGGT GTTTAACCAC GTTTACAACA TCAAGCTGCC AGTGGGATCC	240
CAGTGTTCGG TGGATCTGGA CTCAGCCAGT GGGGAGAAAAG ACCTGGCACC GCCTTCAGAG	300
CCCAGCGAAA GCTTTCAGCA GCACACACTA GATGGGGAAA ACCAGATTGT CTTCACACAT	360
GGCATCAACA TCCCCCGCGG GGCCTGTGGC TGTGCGCGAG CCGCTGATGT TAAGGAGCTG	420
CTGAGCAGAC TGGAGGAGCT GGAGAACCCTG GTGTCTTCCC TGAGGGAGCA ATGTA CTGCA	480
GGAGCAGGCT GCTGTCTCCA GCCTGCCACA GCGCGCTTGG ACACCAGGCC CTCTCTAGC	540
GGTCGGGGCA ACTTCAGCAC TGAAGGATGT GGTGTGTCTT GCGAACCTGG CTGGAAGGCC	600
CCCAACTGCT CTGAGCCCGA ATGTCCAGGC AACTGTCACC TTCGAGGCCG GTGCATTGAT	660
GGGCAGTGCA TCTGTGACGA CCGCTTCACG GCGGAGGACT GCAGCCAGCT GCCTTGCCCC	720
AGCGACTGCA ATGACCAGGG CAAGTGCGTG AATGGAGTCT GCATCTGTTT CGAAGGCTAC	780
GCGGCTGACT GCAGCCGTGA AATCTGCCCC GTGCCCTGCA GTGAGGAGCA CGGCACATGT	840
GTAGATGGCT TGTGTGTGTG CCACGATGGC TTTCGAGGCG ATGACTGCAA CAAGCCTCTG	900
TGTCTCAACA ATTGCTACAA CCGTGGACGA TCGGTGGAGA ATGAGTGCCT GTGTGATGAC	960
GGTTTCACGG GCGAAGACTG CAGTGAGCTC ATCTGCCCCA ATGACTGCTT CGACCGGGGC	1020
CGCTGCATCA ATGGCACCTG CTACTGCCAA GAAGCCTTCA CAGGTGAAGA CTGCGGGAAA	1080
CCCACCTGCC CACATGCCTG CCACACCCAG GCGCGGTGTG AGGAGGGGCA GTGTGTATGT	1140

GATGAGGGCT TTGCCCCGTGT GGACTGCAGC GAGAAGAGGT GTCCCTGCTGA CTGTCACAAAT	1200
CGTGGGCGCT GTGTAGACGG GCGGTGTGAG TGTGATGATG GTTTCAGTGG AGCTGACTGT	1260
GGGGAGCTCA AGTGTCCCAA TGGCTGCAGT GGCCATGGCC GCTGTGTCAA TGGGCAGTGT	1320
GTGTGTGATG AGGGCTATAC TGGGGAGGAC TGCAGCCAGC TACGGTGCCC CAATGACTGT	1380
CACAGTCGGG GCCGCTGTGT CGAGGGCAAA TGTGTATGTG AGCAAGGCTT CAAGGGCTAT	1440
GACTGCAGTG ACATGAGCTG CCTAATGAC TGTACCAGC ACGGCCGCTG TGTGAATGGC	1500
ATGTGTGTTT GTGATGACGG CTACACAGGG GAAGACTGCC GGGATCGCCA ATGCCCCAGG	1560
GACTGCAGCA ACAGGGGCCT CTGTGTGGAC GGACAGTGCC TCTGTGAGGA CGGCTTCACC	1620
GGCCCTGACT GTGCAGAACT CTCTGTCCA AATGACTGCC ATGGCCAGGG TCGCTGTGTG	1680
AATGGGCAGT GCGTGTGCCA TGAAGGATTT ATGGGCAAAG ACTGCAAGGA GCAAAGATGT	1740
CCCAGTGA CTGATGGCCA GGGCCGCTGC GTGGACGGCC AGTGCACTC CCACGAGGGC	1800
TTACAGGCC TGGACTGTGG CCAGCAGTCC TGCCCCAGTG ACTGCAACAA CTTAGGACAA	1860
TGCGTCTCGG GCCGCTGCAT CTGCAACGAG GGCTACAGCG GAGAAGACTG CTCAGAGGTG	1920
TCTCCTCCCA AAGACCTCGT TGTGACAGAA GTGACGGAAG AGACGGTCAA CCTGGCCTGG	1980
GACAAATGAGA TGCGGGTCAC AGAGTACCTT GTCGTGTACA CGCCCAACCA CGAGGGTGGT	2040
CTGGAATGC AGTTCCGTGT GCCTGGGGAC CAGACGTCCA CCATCATCCG GGAGCTGGAG	2100
CCTGGTGTGG AGTACTTTAT CCGTGTATTT GCCATCCTGG AGAACAAGAA GAGCATTCCT	2160
GTCAGCGCCA GGGTGGCCAC GTACTTACCT GCACCTGAAG GCCTGAAATT CAAGTCCATC	2220
AAGGAGACAT CTGTGGAAGT GGAGTGGGAT CCTCTAGACA TTGCTTTTGA AACCTGGGAG	2280
ATCATCTTCC GGAATATGAA TAAAGAAGAT GAGGGAGAGA TCACCAAAAG CCTGAGGAGG	2340
CCAGAGACCT CTTACCGGCA AACTGGCTTA GCTCCTGGGC AAGAGTATGA GATATCTCTG	2400
CACATAGTGA AAAACAATAC CCGGGGCCCT GGCCTGAAGA GGGTGACCAC CACACGCTTG	2460

GATGCCCCCA GGCAGATCGA GGTGAAAGAT GTCACAGACA CCACTGCCCTT GATCACCCTGG	2520
TTCAAGCCCC TGGCTGAGAT CGATGGCATT GAGCTGACCT ACGGCATCAA AGACGTGCCA	2580
GGAGACCGTA CCACCATCGA TGTACACAG GACGAGAACC AGTACTCCAT CGGGAACCTG	2640
AAGCGTGACA CTGAGTACGA GGTGTCCCTC ATCTCCCGCA GAGGTGACAT GTCAGCAAC	2700
CCAGCCAAAG AGACCTTCAC AACAGGCTC GATGCTCCCA GGAATCTTCG ACGTGTTCG	2760
CAGACAGATA ACAGCATCAC COTGGAATGG AGGAATGGCA AGGCAGCTAT TGACAGTTAC	2820
AGAATTAAGT ATGCCCCCAT CTCTGGAGGG GACCACGCTG AGGTTGATGT TCCAAAGAGC	2880
CAACAAGCCA CAACCAAAAC CACACTCACA GGTCTGAGGC CGGGAACCTG ATATGGGATT	2940
GGAGTTTCTG CTGTGAAGGA AGACAAGGAG AGCAATCCAG CGACCATCAA CGCAGCCACA	3000
GAGTTGGACA CGCCCCAAGG CTTTCAGGTT TGTGAAACTG CAGAGACCAG COTGACCCTG	3060
CTCTGGAAGA CACCGTTGGC CAAATTGAC CGCTACCGCC TCAATTACAG TCTCCCCACA	3120
GGCCAGTGGG TGGGAGTGCA GCTTCCAAGA AACACCACTT COTATGTCTT GAGAGGCTG	3180
GAACCAGGAC AGGAGTACAA TGTCTCTCTG ACAGCCGAGA AAGGCAGACA CAAGAGCAAG	3240
CCCCCACGTG TGAAGGCATC CACTGAACAA GCCCCTGAGC TGGAAAACCT CACCGTGAAT	3300
GAGGTGGGCT GGGATGGCCT CAGACTCAAC TGGACCCCGG CTGACCAGGC CTATGAGCAC	3360
TTTATCATTC AGGTGCAGGA GGCCAACAAG GTGGAGGCAG CTCGGAACTT CACCGTGCCT	3420
GGCAGCCTTC GGGCTGTGGA CATACCGGGC CTCAGGCTG CTACGCCTTA TACAGTCTCC	3480
ATCTATGGGG TGATCCAGGG CTATAGAACA CCAGTGCTCT CTGCTGAGGC CTCCACAGGG	3540
GAAACTCCCA ATTGGGAGA GGTCTGGTG GCCGAGGTGG GCTGGGATGC CCTCAAATC	3600
AACCTGACTG CTCCAGAAGG GGCCTATGAG TACTTTTCA TTCAGGTGCA GGAGGCTGAC	3660
ACAGTAGAGG CAGCCCAGAA CCTCACCTC CCAGGAGGAC TGAGGTCCAC AGACCTGCCT	3720
GGGCTCAAAG CAGCCACTCA TTATACCATC ACCATCCGGG GGGTCACTCA GGAATTCAGC	3780

ACAACCCCTC TCTCTGTGA AGTCTTGACA GAGGAGGTTT CAGATATGGG AAACCTCACA	3840
GTGACCGAGG TTAGCTGGGA TGGTCTCAGA CTGAACTGGA CCACGCCAGA TGGAACCTAT	3900
GACCACTTTA CTATTCAGGT CCAGGAGGCT GACCAGGTTG AAGAGGCTCA CAATCTCAGG	3960
GTTCCTGGCA GCCTGGCTTC CATGGAAATC CCAGGCCTCA GGGGTGGCAC TCCTTACACA	4020
GTCAACCTGC ACCGCCAGGT CAGGGGCCAC AGCACTCGAC CCCTTGCTGT AGAGGTCGTC	4080
CAGTGGGACG TGCCGCTCCA GTCCCCGGTG TCGTGAGCTG GGGAAACGACA TCTCCAGCAG	4140
ACAGAGGATC TCCACAGCT GGGAGATTGA GCCGTGTCTG AGGTTGGCTG GGATGGCCTC	4200
AGACTCAACT GGACCCGAGC TGACAATGCC TATGAGCACT TTCTCATTCA GGTCCAGGAG	4260
GTCAACAAAG TGGAGGCAGC CCAGAACCTC ACGTTGCCTG GCAGCCTCAG GGCTGTGGAC	4320
ATCCCCGGCC TCGAGGCTGC CACGCCTTAT AGAGTCTCCA TCTATGGGGT GATCCGGGGC	4380
TATAGAACAC CAGTACTCTC TGCTGAGGCC TCCACAGCCA AAGAACCTGA AATTGGAAAC	4440
TTAAATGTTT CTGACATAAC TCCCGAGAGC TTCAATCTCT CCTGGATGGC TACCGATGGG	4500
ATCTTCGAGA CTTTTACCAT TGAATATTATT GATTCCAATA GGTGCTGGA CACTCTGGAA	4560
TATAATATCT CTGGTGCTGA ACGAACTGCC CATATCTCAG GGCTACCCCC TAGTACTGAT	4620
TTTATTGTCT ACCTCTCTGG ACTTGCTCCC AGCATCCGA CCAAAACCAT CAGTGCCACA	4680
GCCACGACAG AGGCCCTGCC CTTTCTGGAA AACCTAACCA TTCCCGACAT TAATCCCTAC	4740
GGGTTACAG TTTCTGGAT GGCATCGGAG AATGCCTTTG ACAGCTTTCT AGTAACGGTG	4800
GTGGATTCTG GGAAGCTGCT GGACCCCCAG GAATTCACAC TTTCAGGAAC CCAGAGGAAG	4860
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GACAACCTTC TGGTTTCAGA TGCCACCCCA GACGGTTTCC GTCTGTCTG GACAGCTGAT	5040
GAAGGGGTCT TCGACAATTT TTTTCTCAA ATCAGAGATA CCAAAAAGCA GTCTGAGCCA	5100

CTGGAAATAA CCTACTTGC CCCCGAACGT ACCAGGGACA TAACAGGTCT CAGAGAGGCT	5160
ACTGAATACG AAATTGAACT CTATGGAATA AGCAAAGGAA GCGGATCCCA GACAGTCAGT	5220
GCTATAGCAA CAACAGCCAT GCGCTCCCA AAGGAAGTCA TTTTCTCAGA CATCACTGAA	5280
AATTGGGCTA CTGTCAGCTG GAGGGCACCC ACGGCCCAAG TGGAGAGCTT CCGGATTACC	5340
TATGTGCCCC TTACAGGAGG TACACCTCC ATGGTAACTG TGGACGGAAC CAAGACTCAG	5400
ACCAGGCTGG TGAAACTCAT ACCTGGCGTG GAGTACCTG TCAGCATCAT CCGCATGAAG	5460
GGCTTTGAGG AAAGTGAACC TGTCTCAGGG TCATTACCA CAGCTCTGGA TGGCCCATCT	5520
GGCCTGGTGA CAGCCAACAT CACTGACTCA GAAGCCTTGG CCAGGTGGCA GCCAGCCATT	5580
GCCACTGTGG ACAGTTATGT CATCTCTAC ACAGGCGAGA AAGTGCCAGA AATTACCCG	5640
ACGGTGTCCG GGAACACAGT GGAGTATGCT CTGACCGACC TCGAGCCTGC CACGGAATAC	5700
ACACTGAGAA TCTTTGCAGA GAAAGGGCCC CAGAAGAGCT CAACCATCAC TGCCAAGTTC	5760
ACAACAGACC TCGATTCTCC AAGAGACTTG ACTGCTACTG AGGTTCACTC GGAAACTGCC	5820
CTCCTTACCT GCGCACCCCC CCGGGCATCA GTCACCGGT ACCTGCTGGT CTATGAATCA	5880
CTGGATGGCA CAGTCAAGGA AGTCATTGTG GGTCCAGATA CCACCTCCTA CAGCCTGGCA	5940
GACCTGAGCC CATCCACCCA CTACACAGCC AAGATCCAGG CACTCAATGG GCGCCTGAGG	6000
AGCAATATGA TCCAGACCAT CTTCAACCACA ATTGGACTCC TGTACCCCTT CCCCAGGAC	6060
TGCTCCCAAG CAATGCTGAA TGGAGACAGG AGCTCTGGCC TCTACACCAT TTATCTGAAT	6120
GGTGATAAGG CTCAGGCGCT GGAAGTCTTC TGTGACATGA CCTCTGATGG GGGTGGATGG	6180
ATTGTGTTCC TGAGACGCAA AAACGGACGC GAGAACTTCT ACCAAAAGTG GAAGGCATAT	6240
GCTGCTGGAT TTGGGGACCG CAGAGAAGAA TTCTGGCTTG GGCTGGACAA COTGAACAAA	6300
ATCACAGCCC AGGGGCAGTA CGAGCTCCGG GTGGACCTCC GGGACCATGG GGAGACAGCC	6360
TTTGCTGTCT ATGACAAGTT CAGCGTGGGA GATGCCAAGA CTCGCTACAA GCTGAAGGTG	6420

GAGGGGTACA GTGGGACAGC AGGTGACTCC ATGGCCTACC ACAAATGGCAG ATCCTTCTCC	6480
ACCTTTGACA AGGACACAGA TTCAGCCATC ACCAACTGTG CTCTGTCTAC AAGGGGCTTC	6540
TGGTACAGGA ACTGTCACCG TGTCAACCTG ATGGGGAGAT ATGGGGACAA TAACCACAGT	6600
CAGGGCGTTA ACTGGTTCCA CTGGAAGGGC CACGAACACT CAATCCAGTT TGCTGAGATG	6660
AAGCTGAGAC CAAGCAACTT CAGAAATCTT GAAGGCAGGC GCAAACGGGC ATAAATTGGA	6720
GGGACCACTG GGTGAGAGAG GAATAAGGCG GCCCAGAGCG AGGAAAGGAT TTTACCAAAG	6780
CATCAATACA ACCAGCCCAA CCATCGGTCC ACACCTGGGC ATTTGGTGAG AATCAAAGCT	6840
GACCATGGAT CCCTGGGGCC AACGGCAACA GCATGGGCCT CACCTCCTCT GTGATTTCTT	6900
TCTTTGCACC AAAGACATCA GTCTCCAACA TGTTCCTGTT TTGTTGTTTG ATTCAGCAAA	6960
AATCTCCCAG TGACAACATC GCAATAGTTT TTTACTTCTC TTAGGTGGCT CTGGGATGGG	7020
AGAGGGGTAG GATGTACAGG GGTAGTTTGT TTTAGAACCA GCCGTATTTT ACATGAAGCT	7080
GTATAATTAA TTGTCATTAT TTTTGTTAGC AAAGATTAAA TGTGTCATTG GAAGCCATCC	7140
CTTTTTTTAC ATTTCAIACA ACAGAAACCA GAAAAGCAAT ACTGTTTCCA TTTTAAGGAT	7200
ATGATTAATA TTATTAATAT AATAATGATG ATGATGATGA TGAAAACTAA GGATTTTICA	7260
AGAGATCTTT CTTTCCAAAA CATTCTGGA CAGTACCTGA TTGATTTTTT TTTTAAATA	7320
AAAGCACAAG TACTTTTGAA AAAAAA	7346